

mixed POPC and POPS bilayer with molecular dynamics (MD) simulations. One short PLL with more definite conformation and one relative long PLL with random conformation were used for this study. With the simulated systems reached dynamic equilibration, additional 200 ns production MD simulations were performed for each simulated systems. Results demonstrated that the interactions of different length of PLLs with the lipid bilayer directly affect the microscopic properties of a lipid bilayer: lipid head group area, tail order and bilayer thickness, further influencing the mesoscopic properties of the bilayer: electrostatic and mechanical properties of the bilayer. Results also showed that the different types of lipid bilayer can in turn influence the binding dynamics of PLLs with the lipid bilayer. The results will provide molecular insight for the experimental observations about the effect of PLL on cototoxicity and its role in gene delivery, further helping to optimize PLL length and lipid environment for best usage of PLL for its biological applications.

2201-Pos Board B220

Probing the Membrane Bound KCNE1 Protein with Solid State NMR Spectroscopy

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KCNE1, also known as MinK, is a membrane protein that associates with the KCNQ1 channel protein to form a voltage-gated potassium channel. This ion channel is essential to the cardiac action potential that mediates heartbeat and is also critical for potassium ion homeostasis in the inner ear. Dominant mutations in KCNE1 lead to congenital long-QT syndrome and congenital deafness. KCNE1 has been over expressed in *E. coli*, purified into micelles using his-tag affinity chromatography, and reconstituted into POPC/POPG vesicles. ³¹P NMR powder spectra results confirm vesicle formation. Different KCNE1 mutants have been labeled using MTSL, one mutant outside the membrane and the other inside the membrane. By measuring ³¹P relaxation times of the lipids, we can determine the depth that at which KCNE1 is buried inside the vesicles. We also introduced a bicelle system to study the topology of uniform ¹⁵N labeled KCNE1 with respect to the lipid bilayer. By measuring the ¹⁵N NMR signal, we are able to figure out the structural topology of KCNE1 within the lipid bilayer.

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Divalent Cation-Induced PIP2 Clustering in Cholesterol-Containing Membranes: How PIP2 Lateral Distribution affects PIP2-Gelsolin Interaction

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Phosphatidylinositol-(4,5)-bisphosphate (PI(4,5)P₂) is involved in many important cellular events, but the mechanism by which this relatively rare lipid selectively regulates specific proteins is unclear. While many proteins bind to PIP₂ with similar affinities in vitro, only a small subset interact with PIP₂ at specific sites during specific cellular processes. Previously, we showed that lateral distribution of PIP₂ in a background of neutral lipids is affected by divalent cations through counterion-mediated attraction. We further hypothesize that spatial control of PIP₂ concentration can alter the binding of this lipid to its protein targets. To test this hypothesis, we determine if Ca²⁺-induced PIP₂ clustering affects the interaction between PIP₂ and PIP₂-binding proteins with polybasic domains under liquid order/liquid disorder (Lo/Ld) phase demixing conditions.

We first characterize the effect of divalent cations on Lo/Ld phase-demixing. The addition of Ca²⁺ induces a surface pressure drop and lowers the transition surface pressure. In contrast, Mg²⁺ increases the transition surface pressure and has a minimum condensing effect. Topography measurements through AFM show that Ca²⁺-induced PIP₂-rich clusters co-localize with the Lo phase. The effect of PIP₂ microdomain/cluster formation on the regulation of gelsolin was studied using an actin filament severing assay and Ca²⁺-insensitive gelsolin fragments (NiGSN). This functional assay suggests that membrane partitioning of NiGSN is sensitive to PIP₂ local concentration upon phase separation, which also depends on the temperature. Cholesterol-induced phase demixing strongly inhibits the severing function of gelsolin, and the presence of PIP₂ clusters formed by micromolar Ca²⁺ also improves the inhibition efficiency. These observations suggest that changes in local PIP₂ distribution might be a major mechanism to determine how proteins interact with PIP₂ in the membrane. This research may shed a light in studying the interplay between PIP₂, cholesterol and Ca²⁺-signaling.

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Lipid Behavior in Integrin $\alpha 2$ Clustering

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It is becoming increasingly more evident that transmembrane proteins can influence lipid organization in biological membranes. Our previous studies in model membranes have shown that peptides affect the partitioning of lipids in the bilayer. We now want to further investigate these protein-lipid interactions in a more biological context, by looking at integrin clustering in giant plasma membrane vesicles (GMPVs). Integrins are cell surface proteins that traverse the plasma membrane and mediate, for example, cell signaling and virus entry. Binding of Echovirus 1 to integrin $\alpha 2$ induces integrin clustering and the subsequent internalization of the virus-integrin complex. Clustering is crucial for virus entry, but can also be achieved through the addition of integrin specific antibodies to cells. We are interested in the possible reorganization of the membrane upon clustering as well as the influence of plasma membrane lipids on clustering. For this purpose we have prepared GMPVs from SAOS cells expressing $\alpha 2$ integrin, and induced antibody-mediated clustering in them. We are using lipid probes to visualize the liquid ordered and liquid disordered phase, and fluorescent secondary antibodies to detect $\alpha 2$ integrin in the GMPVs with confocal microscopy. We will look at lipid organization in cells expressing integrin mutants, which are unable to cluster and/or internalize. And we will treat the cells and/or GMPVs in ways that alter their lipid content, such as deplete membrane cholesterol with cyclodextrin, and monitor the resulting changes in integrin and lipid behavior in GMPVs. With these experiments we hope to gain further insight into the interplay of transmembrane proteins and lipids in biological membranes. Also, understanding the lipid behavior in integrin clustering will aid in understanding what governs clustering and how it affects membrane architecture. This information can in turn be beneficial for developing ways of preventing virus entry.

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Surface Reflectivity from Absorbed Films of Pulmonary Surfactant at the Air-Liquid Interface

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Pulmonary surfactant (PS) is the complex mixture of lipids and proteins that forms a thin film on the liquid layer that lines the alveolar air-sacks of the lungs. When compressed by the decreasing alveolar surface area during exhalation, the surfactant films reduce surface tension to exceptionally low levels. This behavior in situ contrasts with the performance of spread monolayers in vitro that contain the complete set of surfactant constituents, which collapse promptly when compressed below the equilibrium spreading pressure. The structural characteristics that provide the basis for this functional difference remain controversial. The studies here used X-ray reflectivity to compare the structure of adsorbed films and spread monolayers of extracted calf surfactant on the air-water interface. Our results show the presence in the adsorbed film of additional double layers of distinct electron densities underneath the interfacial monolayer. These results support previous evidence that adsorbed films of pulmonary surfactant have multilamellar thickness, and that the additional layers may have functional significance.

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Thionaphthoquinones Destabilization of Phospholipid Bilayers

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Quinones are structures present in many naturally occurring compounds, e.g. 1,4-naphthoquinones like Vitamin K, doxorubicin, etc. are among the examples of this vast class of chemicals used in the treatment of bleeding, lymphoma, carcinoma, etc. Only one sulfated naphthoquinone was found in nature but many were synthesized and proved to be potent antibacterial and antifungal agents. Furthermore, several thionaphthoquinones have been recently synthesized because of their interesting spectroscopic properties and also as attractive organic dyes due to their high solubility in organic solvents. their red color in the solid state also leads to applications as organic nonlinear optical materials. New thionaphthoquinones and hydroquinones, bearing alkyl side chains that match the phospholipids POPC and POPE, were synthesized in order to investigate their interactions with lipids. It was observed that, in general, these additives destabilize the lipid bilayer and induce less organized structures with higher amount of curvature. Moreover, cubic phases, not normally observed in the pure lipids when fully hydrated, were detected. Coexistence of lamellar phases was interpreted as a consequence of microsegregation of the components in the mixtures